

Patent Application
Attorney Docket No. 3153.00050/PC10299A

AMENDED VERSION

IN THE SPECIFICATION:

Beginning on page 3, line 25 and ending on page 4, line 4, please delete and insert
therefor:

In a preferred embodiment, the culture is inactivated by adding formalin (about 0.5% v/v final concentration). In another preferred embodiment, antigens of the invention are obtained from the supernatant or filtrate of an *E. rhusiopathiae* culture. A culture supernatant or filtrate, in a preferred embodiment, is concentrated about 10-fold and aluminum hydroxide gel (preferably REHYDRAGEL™) is added to the concentrated supernatant or filtrate at a final concentration of about 30% v/v to stabilize the antigen. In another preferred embodiment, a vaccine composition is formulated comprising the antigen and an adjuvant with the adjuvant comprising, for example, about 25% v/v of the vaccine composition. In another preferred embodiment, thimerosal (about 0.01% v/v final concentration) with EDTA (about 0.07% v/v final concentration) are added to the antigens as preservative. A preferred adjuvant, herein referred to as "No.1 Adjuvant", comprises about 2% v/v lecithin, about 18% v/v mineral oil, and about 8% v/v surfactant (e.g., about 5.6% v/v TWEEN 80™ and about 2.4% v/v SPAN 80™), with the remaining volume being a saline solution (e.g., Dulbecco PBS). This adjuvant is described in U.S. Patent Application Serial No. 60/117,705, filed January 29, 1999, entitled "Adjuvants for Use in Vaccines", which is incorporated herein by reference.

Beginning on page 7, line 19 and ending on page 8, line 3, please delete and insert
therefor:

An antigen of the invention may be used in a vaccine composition to immunize an animal. In one embodiment, the vaccine composition contains an antigen of the invention and an adjuvant. In a preferred embodiment, an adjuvant useful for a vaccine composition of the invention comprises a lecithin, an oil, and a surfactant. A vaccine composition formulated with a preferred adjuvant contains a lecithin at from about 0.25% to about 12.5% v/v, more preferably from about 0.5% to about 5%, and most preferably from about 0.5% to about 1.25% v/v, an oil at from about 1% to about 23% v/v, more preferably from about 3.5% to about 10% and most preferably about 4.5%, and an amphiphilic surfactant at from about 1.5% to about 6% v/v, more preferably from about 1.5% to about 4% and most preferably about 2% v/v. Preferably the adjuvant has 2 amphiphilic surfactants, for example TWEEN™ and SPAN™ surfactants, of which one predominantly in the aqueous phase (e.g., TWEEN 80™) of the vaccine composition and one in the oil phase (e.g., SPAN 80™). Preferably, when TWEEN 80™ and SPAN 80™ are used as

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surfactants, the concentration of TWEEN 80™ is about 1½ to about 3 times as high as the concentration of SPAN 80™, preferably about 2 times. A preferred adjuvant contains an aqueous carrier solution, for example, phosphate-buffered saline (PBS) (e.g., Dulbecco PBS). A lecithin and an oil suitable for an adjuvant for the vaccine compositions is a mixture of lecithin in DRAKEOL™ 5 Lt Mineral Oil. Lecithin may be obtained from Central Soya, Fort Wayne, Indiana.
C2 See also U.S. Patent No. 5,084,269, which discusses adjuvant compositions. TWEEN™ and SPAN™ surfactants may be obtained from Van Waters and Rogers, Omaha, Nebraska.

Beginning on page 10, line 27 and ending on line 31, please delete and insert therefor:

C3 *E. rhusiopathiae* strain CN 3342 is cultured in medium containing Difco Proteose Peptone at a concentration of 2.75%, Difco Yeast Extract (0.55%), TWEEN 80™ (0.2%), K₂HPO₄ (0.217%) and KH₂PO₄ (0.061%) in deionized water. The pH of the medium is adjusted to 7.2 with 5N NaOH. The medium is steam sterilized at a minimum of 122° C for 30 to 90 minutes. After autoclaving, sterile 50% dextrose solution is added to a final concentration of 3% w/v.

Beginning on page 12, line 18 and ending on line 27, please delete and insert therefor:

C4 The adjuvant used was No.1 Adjuvant. 1000 mL of No.1 Adjuvant were made from 200 mL filter sterilized lecithin-oil solution (10% lecithin in DRAKEOL™ mineral oil), autoclaved TWEEN 80™ (56 mL) and SPAN 80™ (24 mL), and phosphate buffered saline (Dulbecco PPPBS) (720 mL). The lecithin-oil solution and SPAN 80™ were combined and mixed in a sterile tank for at least 1 hour at room temperature until emulsification was complete. The saline and TWEEN 80™ were combined and mixed in a sterile tank for at least 1 hour at room temperature. The oil mixture was emulsified with the aqueous mixture using a Ross emulsifier. Emulsification was continued by recirculation until all of the adjuvant was added into the saline. The emulsion was then passed twice through a Gaulin press at room temperature. The adjuvant was stored at 2 to 8° C.

Beginning on page 15, line 7 and ending on line 30, please delete and insert therefor:

C5 Sows were bled 0 to 10 days prior to farrowing to determine their *E. rhusiopathiae* antibody titers. Piglets were randomized based on sows' serological titers and farrowing dates. Fifty eight (58) piglets derived from these sows/gilts were bled and vaccinated at approximately 3 weeks of age with one of the two experimental *E. rhusiopathiae* vaccines or the placebo (groups listed in Table 1). At approximately 4 weeks of age the piglets were weaned. At approximately 6